

## Metabolic Responses of Transition Holstein Cows Fed Anionic Salts and Supplemented at Calving with Calcium and Energy<sup>1</sup>

P. Melendez,\* A. Donovan,\* C. A. Risco,\*  
M. B. Hall,† R. Littell,‡ and J. Goff§

\*College of Veterinary Medicine,

†Department of Animal Sciences

and ‡Institute of Food and Agricultural Sciences,  
University of Florida, Gainesville 32610

§National Animal Disease Center,

USDA-Agricultural Research Service, Ames, IA 50010

### ABSTRACT

The objective of this study was to determine the concentrations of plasma Ca, P, Mg, nonesterified fatty acids (NEFA),  $\beta$ -hydroxybutyrate (BHBA), and glucose in transition cows fed anionic salts prepartum and provided with calcium and energy supplements at calving. The study was conducted on a Florida Holstein dairy farm from November to December 1997. Treatments consisted of no treatment ( $n = 30$ ); 60 g of Ca as calcium chloride, orally ( $n = 30$ ); 110 g of Ca as calcium propionate 510 g plus 400 g of propylene glycol, orally ( $n = 30$ ); two doses of 60 g of Ca as calcium chloride, one at calving and the second 24 h later, orally ( $n = 30$ ); and 10 g of Ca as borogluconate, intravenously ( $n = 30$ ). Treatments were administered within 12 h after parturition. Blood samples were collected at d 1 (parturition), 2, 3, 6, 9, and 12 after calving. Plasma total Ca, P, Mg, NEFA, BHBA, and glucose were measured. There were no differences in the concentrations of the blood metabolites among treatments.

**(Key words:** blood metabolite, anionic salt, calcium-energy supplement, transition cow)

**Abbreviation key:** CRD = calving-related disorders, DA = displacement of abomasum, DCAD = dietary cation-anion difference, MF = milk fever, RFM = retained fetal membranes.

### INTRODUCTION

Cows that experience calving-related disorders have significant metabolic changes reflected in blood concentration of some metabolites (Goff and Horst, 1997). Hy-

pocalcemia is characterized by low plasma calcium ( $<7.5$  mg/dl), low plasma phosphorus ( $<2$  mg/dl), and low plasma magnesium concentrations ( $<1.5$  mg/dl [Goff, 1999]). Ketosis and fatty liver are characterized by increased plasma levels of NEFA ( $>1.0$  meq/L), increased plasma ketone bodies concentration ( $>35$  mg/dL), and low plasma glucose ( $<55$  mg/dL [Grummer, 1993; Herdt and Gerloff, 1999]). Some of these metabolic diseases, such as milk fever (MF) and ketosis, have been associated with the incidence of nonmetabolic disorders, such as retained fetal membranes (RFM), metritis, and mastitis (Dohoo and Martin, 1984; Correa et al., 1993). Additionally, some blood metabolite concentrations have been used as potential indicators of disease risk in dairy cattle (Kaneene et al., 1997).

Peripartum plasma calcium levels in cows fed anionic salts from 21 d prepartum until calving are higher than in cows fed diets with a positive dietary cation-anion difference (DCAD). Furthermore, cows fed negative DCAD diets have been shown to experience less mastitis, metritis, and displacement of abomasum (DA) than cows fed no anionic salts (Goff and Horst, 1997; Goff and Horst, 1998a,b). Oral calcium and energy products, such as calcium propionate, increased blood glucose 24 h after treatment and reduced BHBA and NEFA during the first 2 d postpartum in cows that were fed no anionic salts (Goff et al., 1996). However, blood metabolite patterns of cows fed anionic salts and supplemented with calcium or calcium-energy drench at calving have not yet been reported. Our (null) hypothesis was that when anionic salts are fed prepartum, any calcium supplement provided at parturition would not beneficially change metabolic status postpartum. The objective of this study was to determine plasma concentration of calcium, phosphorus, magnesium, NEFA, BHBA, and glucose during the early postpartum period in cows fed anionic diets prepartum and supplemented with calcium and calcium-energy products at calving.

Received August 13, 2001.

Accepted December 5, 2001.

Corresponding author: A. Donovan; e-mail: donovana@mail.vetmed.ufl.edu.

<sup>1</sup>Published as journal series number R-08536 of the Florida Agricultural Experiment Station.

## MATERIALS AND METHODS

### Cows and Herd Management

The study was conducted on a commercial dairy farm with 3600 Holstein milking cows located in north central Florida, with a rolling herd average milk production of 10,500 kg. Most lactating cows were housed in a dry-lot system and fed the same TMR three times a day, except postpartum transition cows, which received a diet higher in effective NDF. Close-up prepartum transition cows were housed and managed in a dry-lot system; actual days in prepartum lot were 22.5 (range 15 to 68). Twice a day, they received a diet with DCAD of  $-80 \text{ mEq/kg DM}$  using the equation  $\text{DCAD (mEq/kg DM)} = (\text{Na} + \text{K}) - (\text{Cl} + \text{S})$  (Goff and Horst, 1998a). At calving, the calf was immediately separated from the dam. If the cow needed calving assistance, she was moved to a maternity barn. Cows were processed within 12 h postpartum, on a routine basis, which included recording BCS, udder score (for edema presentation), reproductive tract status (trauma or lacerations), and whether the cow was suspected of having RFM. If cows developed either RFM or MF, they were treated with local or systemic antibiotics and calcium salts intravenously and remained in the hospital barn until recovery. After postpartum processing, cows were moved to a postpartum lot and fed a diet higher in effective NDF to prevent DA development. Any cow with a decrease in daily milk yield was moved from the milking herd to the hospital barn to be examined and treated as needed. Cows were milked three times a day in the milking herd and twice a day at the hospital barn.

### Experimental Protocol

During November to December 1997, 150 parturient cows were matched by parity (1, 2, 3+) and allocated using a computer-generated randomization to five different experimental treatments, using commercially available products according to label directions. Matching was done by assigning randomly to each treatment group a similar number of cows of parity 1. The same was done for parity 2 and 3+. This ensured equal distribution of treatments within parity.

Any cows with induced parturition or cesarean section were excluded. Group 1 consisted of 30 cows receiving no treatment at parturition. Group 2 consisted of 30 cows receiving 60 g of calcium as calcium chloride paste (Super Calcium Gel; RXV, Kansas City, MO), orally. Group 3 consisted of 30 cows receiving 110 g of calcium as calcium propionate (510), plus 400 g of propylene glycol (Dr. Larson's Up&Over 1000; Veterinary Concepts, Spring Valley, WI), orally. Group 4 consisted of 30 cows receiving two doses of 60 g of calcium

as calcium chloride paste (Super Calcium Gel), orally, one within 12 h after calving and the second dose 24 h later. Group 5 consisted of 30 cows receiving 10 g of calcium as calcium gluconate (RXV), intravenously. With the exception of the second calcium chloride treatment in group 4, all treatments were administered within 12 h after parturition.

Blood samples were taken in all cows from the coccygeal vein, using an evacuated tube (Vacutainer; Becton Dickinson, Rutherford, NJ). Samples were collected on the day of parturition (d 1) before cows received any treatment and on d 2, 3, 6, 9, and 12 after calving at the same time of the day. Previous studies have documented mineral and energy status up to d 7 postpartum (Risco et al., 1994; Goff et al., 1996). We were interested in determining whether or not there was a longer-term effect of treatments at calving on blood metabolites, beyond 1 wk postpartum. The sampling protocol was established according to the allowance of the commercial conditions of the farm. Samples were taken in heparinized tubes for plasma metabolite analysis and in NaFl tubes for glucose analysis.

### Laboratory Analysis

Samples were centrifuged at 4000 rpm for 10 min. Plasma was separated and stored in plastic tubes and frozen at  $-20^{\circ}\text{C}$  until analysis was performed. All samples were sent to and processed at National Animal Disease Center, Agricultural Research Service, USDA, Ames, Iowa. Plasma total calcium, phosphorus, magnesium, NEFA, BHBA, and glucose were measured. Plasma total calcium, and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer Corp., 1965). Plasma concentration of phosphorus was determined colorimetrically (Parekh and Jung, 1970). NEFA were determined by an enzymatic-colorimetric method (Johnson and Peters, 1993), with a commercial kit (NEFA-C kit; WAKO, Saitama, Japan). BHBA was determined by an enzymatic-colorimetric method (Williamson and Mellanby, 1974), with a commercial kit (Sigma beta-BHA kit # 310-A; Sigma, St. Louis, MO). Glucose was determined with a kit based on Trinder reaction (Sigma, St. Louis, MO [Bergmeyer and Bernt, 1974]). Colorimetric assays were performed in 96-well microtiter plates, and absorbance was read without knowledge of treatments using a microplate reader (Thermomax Microplate Reader; Molecular Devices, Sunnyvale, CA).

### Statistical Analyses

Concentration of blood metabolites during the first 12 d postpartum was an outcome variable. Results were

analyzed with repeated measures analysis developing a mixed linear model, using the mixed procedure in SAS 7.0 (SAS, 1999). Time was treated as a continuous variable. Polynomial effects were modeled to obtain smoothed trends over time. Significance was declared at  $P \leq 0.05$ . Mixed models were defined as:

$$Y_{ilk} = \mu + T_i + \text{Cow}(T_i) + \text{Day}_k + (\text{Day} \times \text{Day}) + (\text{Day} \times \text{Day} \times \text{Day}) + P_1 + (P \times T)_{li} + (P \times \text{Day}) + (P \times \text{Day} \times \text{Day}) + (P \times \text{Day} \times \text{Day} \times \text{Day}) + (\text{Day} \times T)_{ki} + (\text{Day} \times \text{Day} \times T) + (\text{Day} \times \text{Day} \times \text{Day} \times T) + (P \times T \times \text{Day})_{lik} + e_{lik}$$

Where:

- $Y_{ilk}$  = blood metabolite,
- $T_i$  = fixed effect of treatment,
- $\text{Cow}(T_i)$  = random effect of cow nested in treatment,
- $\text{Day}_k$  = fixed linear effect of time,
- $(\text{Day} \times \text{Day})$  = fixed quadratic effect of time,
- $(\text{Day} \times \text{Day} \times \text{Day})$  = fixed cubic effect of time,
- $P_1$  = fixed effect of parity,
- $(P \times T)_{li}$  = fixed effect of interaction parity and treatment,
- $(P \times \text{Day})_{lk}$  = fixed effect of interaction parity and time,
- $(P \times \text{Day} \times \text{Day})$  = fixed effect of interaction parity and quadratic effect of time,
- $(P \times \text{Day} \times \text{Day} \times \text{Day})$  = fixed effect of interaction parity and cubic effect of time,
- $(\text{Day} \times T)_{ik}$  = fixed effect of interaction time and treatment,
- $(\text{Day} \times \text{Day} \times T)$  = fixed effect of interaction of quadratic effect of time and treatment,
- $(\text{Day} \times \text{Day} \times \text{Day} \times T)$  = fixed effect of interaction of cubic effect of time and treatment,
- $(P \times T \times \text{Day})_{lik}$  = fixed effect of interaction time, treatment, and parity, and
- $e_{ilk}$  = random error term.

For all models, the best fit was given by the autoregressive covariance structure, based on Schwarz's Bayesian Criterion. The larger the value of Schwarz's Bayesian Criterion, the better the structure of the model (Littell et al., 1998).

## RESULTS AND DISCUSSION

### Calcium

There were no significant effects of treatment or any interactions involving treatment, nor was there an ef-

fect of parity (Table 1). Only differences in the concentration of Ca over time (linear and cubic effects of day; see Table 1 and Figure 1) and the interactions of parity  $\times$  day and parity  $\times$  day  $\times$  day were found. The change in plasma concentration of Ca over time has been well documented (Goff and Horst, 1997; Goff and Horst, 1998a; Goff, 1999). The interaction between parity and day indicated that on some days older cows presented lower Ca concentrations than younger animals. The relationship between age and hypocalcemia has also been well documented (Goff and Horst, 1997, 1998a). Of the total number of cows, 34.6% had a Ca concentration  $<7.5$  mg/dl before receiving any treatment at calving. These findings are in contrast to Goff et al. (1996), who reported that subclinical hypocalcemia (Ca  $<7.5$  mg/dl) affects approximately 50% of all adult dairy cattle. Clinical signs of MF are not seen until calcium is about 4 mg/dl (Goff, 1999), and no cows had levels  $<4.5$  mg/dl in the present experiment. Adequate management of anionic diets before calving may have reduced the incidence of hypocalcemia in the present study. Cows fed anionic diets prepartum showed higher levels of plasma calcium postpartum than cows fed no anionic salts (Joyce et al., 1997). In accordance with these findings is the very low incidence of MF reported at the farm during that season. Furthermore, the lack of treatment effect on plasma Ca might be explained because we were obtaining blood samples every 24 h. Plasma Ca levels in cows supplemented with either intravenous calcium borogluconate or calcium chloride and calcium propionate orally rise dramatically within the first 30 to 60 min after treatment and then decline slowly to base levels within the next 6 to 8 h (Goff and Horst, 1993; Goff and Horst, 1998b). In light of these results, calcium supplementation at parturition as a preventive measure may not be necessary under good management of negative DCAD diets.

### Phosphorus

There were no significant effects of treatment or any interactions involving treatment except for day  $\times$  treatment ( $P < 0.05$ ; see Table 1). Parity, linear, quadratic, and cubic effects of day, and linear and quadratic interactions of day with parity, were significant ( $P < 0.05$ ; see Table 1). Before receiving any treatment, 68.8% of the cows on d 1 had P concentrations  $<4$  mg/dl with a mean and standard error of  $3.66 \pm 0.10$  mg/dl. Only 11% showed concentrations lower than 2 mg/dl. Phosphorus concentrations on d 1 were in agreement with other trials (Joyce et al., 1997; Goff and Horst, 1998b; Dhiman and Sasidharan, 1999). At d 2, only 41% of the cows had P levels  $<4$  mg/dl, and 8% had P levels  $<2$  mg/dl. Normal values of P concentrations should be between

**Table 1.** ANOVA table. Repeated measures. Mixed models for total plasma calcium, phosphorus, and magnesium.

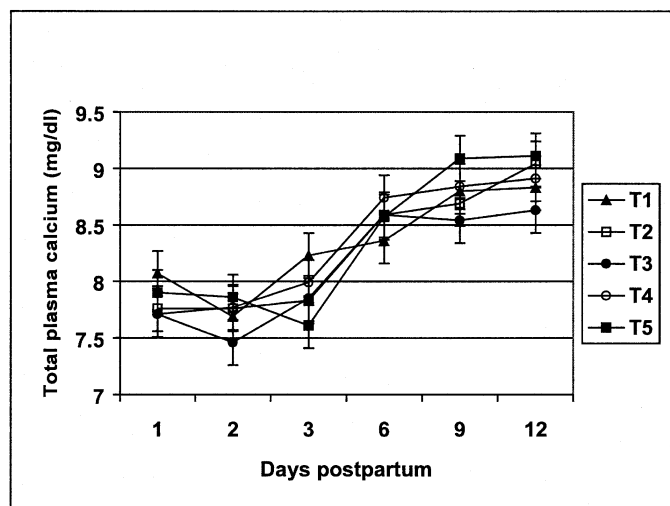
| Source of variation                              | Calcium | Phosphorus | Magnesium |
|--|---------|------------|-----------|
| Treatment  | NS      | NS         | NS        |
| Parity   | NS      | *          | *         |
| Parity $\times$ treatment                        | NS      | NS         | NS        |
| Day  | *       | *          | *         |
| Day $\times$ day                                 | *       | *          | *         |
| Day $\times$ day $\times$ day                    | *       | *          | NS        |
| Day $\times$ treatment                           | NS      | *          | NS        |
| Day $\times$ day $\times$ treatment              | NS      | NS         | NS        |
| Day $\times$ day $\times$ day $\times$ treatment | NS      | NS         | NS        |
| Day $\times$ parity                              | *       | *          | NS        |
| Day $\times$ day $\times$ parity                 | *       | *          | *         |
| Day $\times$ day $\times$ day $\times$ parity    | NS      | NS         | *         |
| Day $\times$ parity $\times$ treatment           | NS      | NS         | NS        |

\* $P \leq 0.05$ .NS:  $P > 0.05$ .

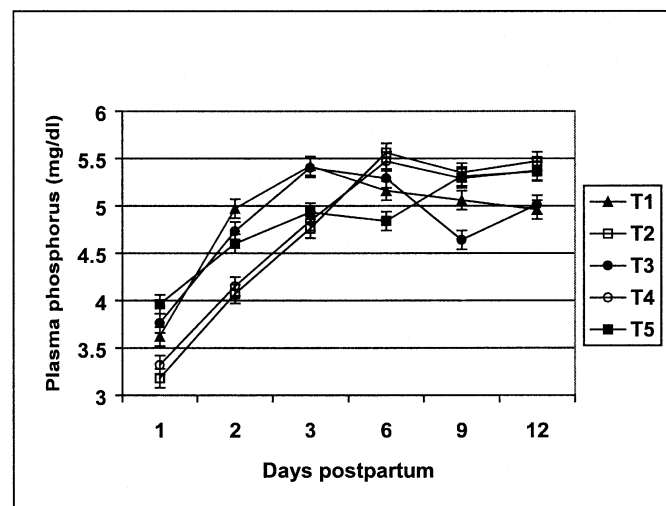
4 and 8 mg/dl in adult animals (Goff, 1999). In our study, mean phosphorus levels ( $4.54 \pm 0.01$ ) recovered to  $\geq 4$  mg/dl at d 2, independent of any treatment (Figure 2). Because calcium levels in this experiment were relatively normal and not affected by treatment and probably were the response to an adequate use of anionic salts, the same conclusion can be suggested for phosphorus. However, the effect of DCAD with respect to changes in P have been inconsistent (Joyce et al., 1997; Vagnoni and Oetzel, 1998) and might be a secondary effect as a result of phosphorus homeostasis being closely related to calcium metabolism (Goff, 1999). Furthermore, the close-up diet offered to these cows contained 0.35% P (DM basis), which is an adequate level

for this physiological stage (Goff, 2000). When normocalcemia is restored, PTH (parathyroid hormone) secretion is lowered, reducing urinary and salivary loss of P, and reactivating the absorption of dietary P and the reabsorption of salivary P secretions (Goff, 1999). Additionally, Dhiman and Sasidharan (1999) did not find a treatment effect on P concentrations in cows supplemented with calcium chloride.

In our trial, there was a cubic effect of P concentration by day, and older cows had higher P concentrations. These patterns have been well documented (Goff and Horst, 1998b). Interaction of day  $\times$  treatment was significant, indicating that curves are not parallel over time. Phosphorus concentrations were lower in cows

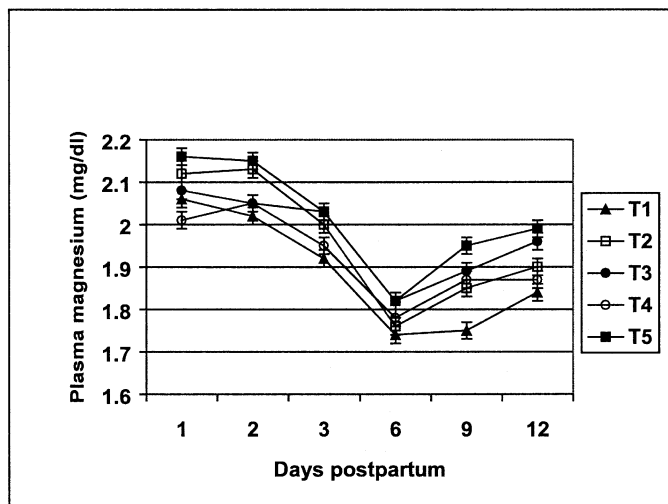


**Figure 1.** Total plasma calcium (mean and SEM) in transition cows fed anionic salts under different treatment protocols. T1: control; T2:  $\text{CaCl}_2$ ; T3: Ca propionate plus propylene glycol; T4:  $\text{CaCl}_2$ , 2 doses; T5: Ca gluconate. Treatment effects not significant; linear, quadratic, and cubic effects of day ( $P < 0.05$ ).



**Figure 2.** Plasma phosphorus (mean and SEM) in transition cows fed anionic salts under different treatment protocols. T1: control; T2:  $\text{CaCl}_2$ ; T3: Ca propionate plus propylene glycol; T4:  $\text{CaCl}_2$ , 2 doses; T5: Ca gluconate. Treatment effects not significant; linear, quadratic, and cubic effects of day; day  $\times$  treatment interaction ( $P < 0.05$ ).





**Figure 3.** Plasma magnesium (mean and SEM) in transition cows fed anionic salts under different treatment protocols. T1: control; T2:  $\text{CaCl}_2$ ; T3: Ca propionate plus propylene glycol; T4:  $\text{CaCl}_2$ , 2 doses; T5: Ca gluconate. Treatment effects not significant; linear and quadratic effects of day ( $P < 0.05$ ).

treated with one dose of  $\text{CaCl}_2$  at d 2 and 3, but the levels recovered and equalized to the other groups beyond d 6 (Figure 2). These interactions are highly complex and difficult to explain and might be normal physiological responses.

### Magnesium

There were no significant effects of treatment or any interactions involving treatment (Table 1). Parity, linear and quadratic effects of day, and quadratic and cubic interactions of day with parity were significant ( $P < 0.05$ ; see Table 1). Only 18% of the cows at d 1 and only 13% at d 2 had levels less than 1.8 mg/dl. Normal levels of plasma magnesium are 1.8 to 2.4 mg/dl (Goff, 1999). As Figure 3 shows, the curves are clearly parallel over time and conclusive, expressing the lack of treatment effect. Only day and parity were significant variables in this model. A quadratic effect was described over time. Day  $\times$  day  $\times$  parity and day  $\times$  day  $\times$  day  $\times$  parity effect interactions were also significant. Levels decreased until d 6, with an average and standard error of the means of  $1.77 \pm 0.02$ . Afterward, levels increased to  $1.90 \pm 0.02$  at d 12. Goff and Horst (1998b) reported a similar pattern of plasma magnesium, but the levels were higher at calving and at 1 d postpartum (mean = 2.6 mg/dl) than our findings. Additionally, Dhiman and Sasidharan (1999) found that serum Mg concentrations remained low when cows were treated with calcium chloride with a similar tendency reported in the present study. One explanation may be that precalving transi-

tion cows fed negative DCAD diets should have higher calcium levels than positive DCAD diets; in this case, the higher the levels of calcium, the lower the PTH secretion and the higher the urine Mg excretion (Goff et al., 1996).

### NEFA

There were no significant treatment or parity effects or any interactions involving those terms. Only day was a significant predictor of NEFA concentration with linear and cubic effects ( $P < 0.05$ ) described over time (Table 2, Figure 4). At d 1, 45.4% of the cows had a NEFA level  $>1.0$  mEq/L, before receiving any treatment. Normal values should be between 0.7 to 0.9 mEq/L at parturition; values greater than this indicate excessive fat mobilization (Studer et al., 1993; Goff et al., 1996; Kaneene et al., 1997).

The acute increase of plasma NEFA at calving is a consequence of decreased DMI before calving and increased plasma lipolytic hormones related to parturition (Grummer, 1993). A higher rate of fat mobilization is typically in response to energy demands (negative energy balance) and is related to a lower DMI occurring before parturition (Gerloff and Herdt, 1999). In this sense, almost half of the cows in our experiment had excessive fat mobilization, which places them at higher risk of fatty liver development. A lower prepartum DMI due to the addition of anionic salts, stress, and feed management could explain these results. Anionic salts are highly unpalatable (Goff and Horst, 1998a). As Figure 4 shows, the curves are similar, indicating lack of treatment effect with a very well-defined pattern over time. In our study, we found high levels of NEFA at calving, with a slow decline beginning around 3 d postpartum. Other studies have also reported that NEFA levels start to increase within the last 5 d before parturition, with the highest levels at calving or the day before and a slow decline around 3 to 5 d postpartum (Grummer, 1993; Studer et al., 1993; Goff et al., 1996).

Goff et al. (1996) did not find an effect of calcium propionate supplementation at calving on plasma NEFA concentration in Holstein cows, but they found a positive effect in Jersey cows. However, in studies where propylene glycol was supplemented for several days before parturition, the plasma NEFA levels were lower than the control groups (Studer et al., 1993; Grummer et al., 1994). Again, a logical explanation for these contrasts may be that NEFA levels at parturition are defined before calving, and only one dose of propionate and propylene glycol is not sufficient to affect the fat mobilization when cows are in negative energy balance.

**Table 2.** ANOVA table. Repeated measures. Mixed models for plasma NEFA, BHBA, and glucose.

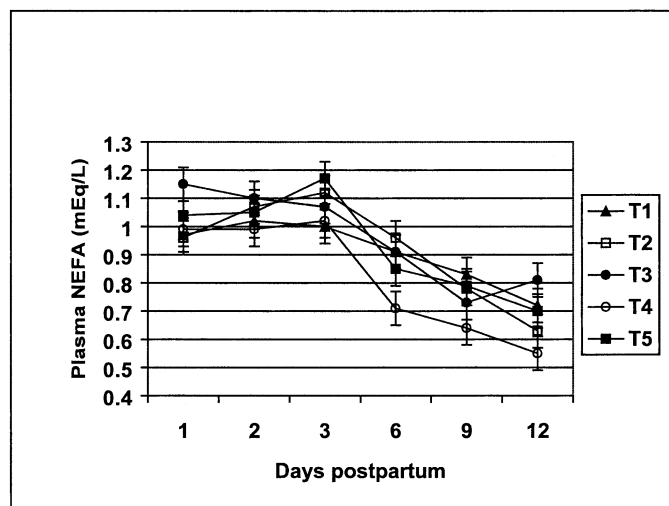
| Source of variation                              | NEFA | BHBA | Glucose |
|--|------|------|---------|
| Treatment  | NS   | NS   | NS      |
| Parity   | NS   | NS   | *       |
| Parity $\times$ treatment                        | NS   | NS   | NS      |
| Day  | *    | *    | *       |
| Day $\times$ day                                 | NS   | NS   | *       |
| Day $\times$ day $\times$ day                    | *    | NS   | *       |
| Day $\times$ treatment                           | NS   | NS   | NS      |
| Day $\times$ day $\times$ treatment              | NS   | NS   | NS      |
| Day $\times$ day $\times$ day $\times$ treatment | NS   | NS   | NS      |
| Day $\times$ parity                              | NS   | NS   | NS      |
| Day $\times$ day $\times$ parity                 | NS   | NS   | NS      |
| Day $\times$ day $\times$ day $\times$ parity    | NS   | NS   | NS      |
| Day $\times$ parity $\times$ treatment           | NS   | NS   | NS      |

\* $P \leq 0.05$ .NS:  $P > 0.05$ .

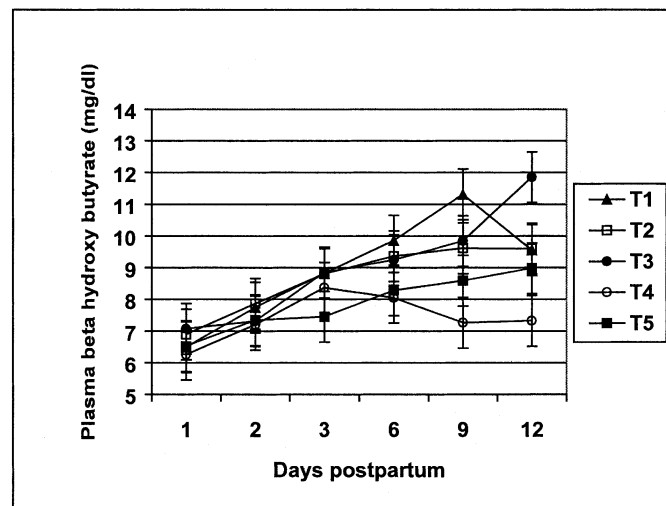
### $\beta$ -hydroxybutyrate

There were no significant treatment or parity effects or any interactions involving those terms. Only day was a significant predictor of NEFA concentration with a linear effect ( $P < 0.05$ ) over time (Table 2, Figure 5). Overall, all the cows had a mean and standard error of the means of  $6.60 \pm 0.40$  mg/dl before receiving any treatment. No animals had a concentration  $>20$  mg/dl at parturition. Only 5.8% of the animals had BHBA levels  $>25$  mg/dl beyond d 6 postpartum, which could indicate clinical ketosis (Figure 5). These values are not in agreement with Goff et al. (1996), in which cows receiving calcium propionate at calving had significantly lower levels of BHBA ( $4.4 \pm 0.40$  mg/dl) than the

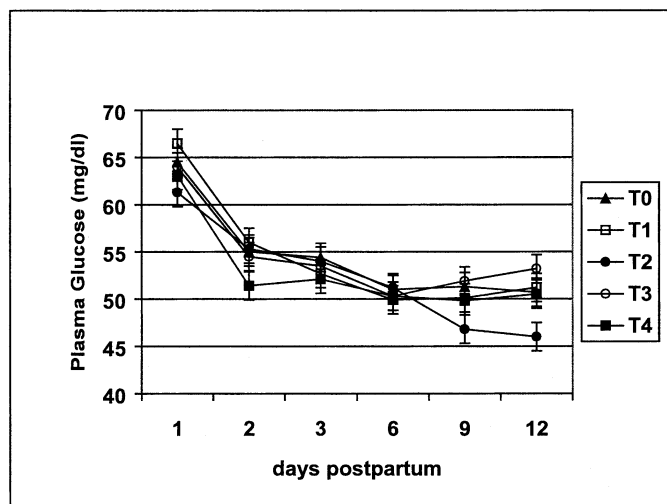
control group, 24 h after treatment. However, they used two doses of calcium propionate, once at calving and again 12 h later. By 10 d postpartum the levels of BHBA were not statistically different between treated and control groups. In contrast, although there was no significant effect of treatment or treatment  $\times$  day interaction in the present study, some least square means combinations of treatment  $\times$  day interaction were significant (Figure 5). Although some studies (Studer et al., 1993; Grummer et al., 1994) have demonstrated a positive effect of propylene glycol fed for several days prepartum, our results suggests that one dose of propionate and propylene glycol would not be sufficient to obtain decreased levels of BHBA and differences among groups.



**Figure 4.** Plasma NEFA (mean and SEM) in transition cows fed anionic salts under different treatment protocols. T1: control; T2:  $\text{CaCl}_2$ ; T3: Ca propionate plus propylene glycol; T4:  $\text{CaCl}_2$ , 2 doses; T5: Ca gluconate. Treatment effects not significant; linear and cubic effects of day ( $P < 0.05$ ).



**Figure 5.** Plasma BHBA (mean and SEM) in transition cows fed anionic salts under different treatment protocols. T1: control; T2:  $\text{CaCl}_2$ ; T3: Ca propionate plus propylene glycol; T4:  $\text{CaCl}_2$ , 2 doses; T5: Ca gluconate. Treatment effects not significant; linear effect of day ( $P < 0.05$ ).



**Figure 6.** Plasma glucose (mean and SEM) in transition cows fed anionic salts under different treatment protocols. T1: control; T2:  $\text{CaCl}_2$ ; T3: Ca propionate plus propylene glycol; T4:  $\text{CaCl}_2$ , 2 doses; T5: Ca gluconate. Treatment effects not significant; linear, quadratic, and cubic effects of day ( $P < 0.05$ ).

## Glucose

There were no significant treatment effects or any interactions involving treatment. Parity was significant ( $P < 0.05$ ), but not interactions involving parity. There were linear, quadratic, and cubic effects of day ( $P < 0.05$ ; see Table 2 and Figure 6). There was no difference among glucose levels on the first day. Paradoxically, cows receiving propionate plus propylene glycol had lower levels of glucose than the other groups at d 9 and 12. Furthermore, as Figure 5 shows, there were higher levels of BHBA at d 12 for group 3 than the rest of the treatments, which is consistent with the lower levels of glucose for the same group during the same day (Figure 6). We do not have an explanation for this finding. The possibility that cows receiving propylene glycol plus calcium propionate produced more milk during the first 10 d postpartum, experiencing low levels of glucose and higher levels of BHBA, may be likely. Unfortunately, we could not measure milk production during the first 10 d of lactation; however, using daily milk yield data, milk yield between d 10 and d 300 in lactation was not different among groups ( $P > 0.05$  [unpublished data]).

## CONCLUSIONS

Calcium-energy and calcium supplements at parturition in general did not affect the overall plasma calcium, phosphorus, magnesium, NEFA, BHBA, glucose concentrations and milk yield in Holstein cows fed anionic salts during the last 21 d of gestation.

## ACKNOWLEDGMENTS

This research was supported by North Florida Holsteins, Inc. (Bell, FL), Veterinary Concepts (Spring Valley, WI), and the University of Florida (Gainesville, FL).

## REFERENCES

- Bergmeyer, H. U., and E. Bernt. 1974. Pages 1205–1212 in *Methods of Enzymatic Analysis*. Verlag Chemie. 2nd ed. Weinheim, Germany.
- Correa, M. T., H. Erb, and J. Scarlett. 1993. Path analysis for seven postpartum disorders of Holstein cows. *J. Dairy Sci.* 76:1305–1312.
- Dhiman, T. R., and V. Sasidharan. 1999. Effectiveness of calcium chloride in increasing blood calcium concentrations of periparturient dairy cows. *J. Anim. Sci.* 77:1597–1605.
- Dohoo, I. R., and S. W. Martin. 1984. Disease, production and culling in Holstein-Friesian cows. IV. Effects of disease on production. *Prev. Vet. Med.* 2:755–770.
- Gerloff, B. J., and T. H. Herdt. 1999. Fatty liver in dairy cattle. Pages 230–233 in *Current Veterinary Therapy 4. Food Animal Practice*. J. Howard and R. Smith, ed. W. B. Saunders Company, Philadelphia, PA.
- Goff, J. P. 2000. Determining the mineral requirement of dairy cattle. Pages 106–132 in *Proc. 11th Annual Florida Ruminant Nutrition Symposium*, University of Florida, Gainesville, FL.
- Goff, J. P. 1999. Treatment of calcium, phosphorus, and magnesium balance disorders. *Vet. Cl. North Amer. Food Anim. Pract.* 15:619–639.
- Goff, J. P., and R. L. Horst. 1998a. Factors to concentrate on to prevent periparturient disease in the dairy cow with special emphasis on milk fever. Pages 154–163 in *Proc. 31st Conf. of American Association of Bovine Practitioners*, Spokane, WA.
- Goff, J. P., and R. L. Horst. 1998b. Use of hydrochloric acid as a source of anions for prevention of milk fever. *J. Dairy Sci.* 81:2874–2880.
- Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.* 80:1260–1268.
- Goff, J. P., R. L. Horst, P. W. Jardon, C. Borelli, and J. Wedam. 1996. Field trials of an oral calcium propionate paste as an aid to prevent milk fever in periparturient dairy cows. *J. Dairy Sci.* 79:378–383.
- Goff, J. P., and R. L. Horst. 1993. Oral administration of calcium salts for treatment of hypocalcemia in cattle. *J. Dairy Sci.* 76:101–108.
- Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci.* 76:3882–3896.
- Grummer, R. R., J. C. Winkler, S. J. Bertics, and V. A. Studer. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. *J. Dairy Sci.* 77:3618–3623.
- Herdt, T. H., and B. J. Gerloff. 1999. Ketosis. Pages 226–230 in *Current Veterinary Therapy 4. Food Animal Practice*. J. Howard and R. Smith, ed. W. B. Saunders Company, Philadelphia, PA.
- Johnson, M. M., and J. P. Peters. 1993. Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma. *J. Anim. Sci.* 71:753–756.
- Joyce, P. W., W. K. Sanchez, and J. P. Goff. 1997. Effect of anionic salts in prepartum diets based on alfalfa. *J. Dairy Sci.* 80:2866–2875.
- Kaneene, J. B., R. A. Miller, T. H. Herdt, and J. C. Gardiner. 1997. The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. *Prev. Vet. Med.* 31:59–72.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216–1231.
- Parekh, A. C., and D. H. Jung. 1970. Serum inorganic phosphorus determination using p-phenylenediamine as a reducing agent. *Clin. Chim. Acta* 27:373–377.
- Perkin-Elmer Corp. 1965. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer Corp., Norwalk, CT.

- Risco, C. A., M. Drost, W. W. Thatcher, J. Savio, and M. J. Thatcher. 1994. Effects of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postpartum dairy cows. *Theriogenology* 42:183–203.
- SAS. 1999. SAS/STAT Software: Change and enhancements through release 7.0 for windows. SAS Inst. Inc., Cary, NC.
- Studer, V. A., R. R. Grummer, S. J. Bertics, and C. K. Reynolds. 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows. *J. Dairy Sci.* 76:2931–2939.
- Vagnoni, D. B., and G. R. Oetzel. 1998. Effects of dietary cation-anion difference on the acid-base status of dry cows. *J. Dairy Sci.* 81:1643–1652.
- Williamson, D. H., and J. Mellanby. 1974. D-3-hydroxybutyrate. Page 1836 in *Methods of Enzymatic Analysis*. H. U. Bergmeyer, ed. Academic Press, London.